

but cannot merge together to form a continuous filament. Since each Rad51 monomer covers three bases/basepairs, the naked DNA between two adjacent filament patches could be too short to accommodate another protein monomer. Moreover, dsDNA has no directionality, meaning that the filaments can grow in both directions, thus two filaments can grow in opposite directions resulting in them two being out-of-phase at their point of contact. Supporting our hypothesis, when filaments are formed at low protein concentration, which result a lower number of filaments per DNA, we see less rigid regions. The results also demonstrate the general applicability of nanochannels for studies of DNA-protein complexes at extremely low forces not easily obtained using other techniques.

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A Single-Strand Annealing Protein Clamps DNA to Detect Homology

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DNA repair of double-strand breaks is essential for genome maintenance. Among other mechanisms, the repair is mediated by homologous recombination. The recombination pathway of single-strand annealing is promoted by proteins like the eukaryotic RAD52 or the related viral protein Red β , which are thought to act as multimeric ring-shaped complexes. However, it is unclear how a ring-like structure promotes annealing on the molecular level and recognizes homology. By single-molecule experiments, we show that annealing is driven by Red β monomers. Red β monomers weakly hold single DNA strands together. Dimerization of Red β on complementary DNA strands triggers the capture of the homologue and leads to nucleoprotein filament growth. Interestingly, this dimerization strongly clamps DNA, and subsequent filament formation is accompanied by a structural change of Red β . Due to biochemical, structural, and functional similarities of single-strand annealing proteins, our proposed DNA clamping may point at a general molecular recombination mechanism for genome maintenance.

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Kinetic Analysis of Interactions between MutS, MutL and DNA During Initiation of DNA Mismatch Repair

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Mismatch repair (MMR) is essential for correcting base-pairing errors in DNA. MutS protein recognizes mis-paired bases in DNA and recruits MutL to signal excision and re-synthesis of the mismatched strand. The mechanism of MMR is under active investigation—especially the transient events involved in mismatch recognition and initiation of repair. We are utilizing transient kinetic methods coupled with fluorescence spectroscopy to determine the mechanisms of action of MutS and MutL from *Thermus aquaticus*. We have built a kinetic model of the ATPase-linked actions of MutS on DNA whereby initial rapid encounter between the two establishes a weak binding equilibrium ($KD1 = 5 \mu M$), followed by slow formation of a mismatch-specific MutS-DNA complex with the DNA in bent conformation ($k_{conf} \sim 30 s^{-1}$ and $KD2 = 5 nM$). ATP binding to MutS-DNA complex ($k_{ON} = 0.5 \mu M^{-1}s^{-1}$) is followed by two slow steps involving conformational changes in MutS that correspond to unbending of DNA at the mismatch site ($\sim 3 s^{-1}$), and subsequent MutS release from the mismatch ($\sim 0.5 s^{-1}$).

To understand the mechanism of MMR during the transition between mismatch recognition and initiation of excision, we plan kinetic analysis of the concerted actions of MutS and MutL on DNA. We are developing fluorescence-based assays to monitor MutL interactions with DNA and with the MutS-DNA complex. Currently, we are testing the fluorescent nucleoside analog 6-methyl-isoxanthopterin (6-MI), paired with cytosine and located adjacent to a mismatch. This reporter can be used to analyze MutS dynamics at the mismatch site and its communication with MutL, thus enabling novel mechanistic insights into the mechanism of initiation of MMR.

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Building a Better Engine: Stimulation of Single Molecules of SGS1 by RPA and Top3-RMI1

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Sgs1 is the Bloom's Syndrome helicase (BLM) ortholog and the sole RecQ-family helicase in *Saccharomyces cerevisiae*. Genetic defects in Sgs1 cause

gross chromosomal rearrangements and in humans manifest as Bloom's Syndrome, a disease associated with accelerated onset of sporadic cancers. Early during homologous recombination-mediated repair of dsDNA breaks, Sgs1 stimulates long-range resection in coordination with Mre11/Rad50/Xrs2 and the helicase/endonuclease Dna2. Later during recombination, Sgs1 forms the core of the Holliday junction (HJ) dissolution complex with Top3 (type-I topoisomerase) and Rmi1. In contrast to other members of the RecQ-family, Sgs1 is a remarkably active helicase; however, the mechanism by which Sgs1 alternates between resection and HJ dissolution remain unclear—in part due to outstanding questions regarding properties of the core helicase activity. We combined optical trapping, microfluidics and fluorescence microscopy to visualize binding and translocation of Sgs1 on DNA. Sgs1 binds internally along dsDNA and association is enhanced by RPA and Top3-Rmi1. We observe complex translocation phenomenon including pauses, reversals and rate changes. In the presence of RPA, Sgs1 translocates at a rate of 200-350 bp/sec, traveling 1,200 bp, on average. Addition of Top3-Rmi1 doubles both the rate and translocation distance. To distinguish between DNA translocation and unwinding, we used TIRF microscopy to visualize the accumulation of fluorescent RPA on ssDNA. In the absence of salt, Sgs1 is a potent helicase, rapidly and completely unwinding lambda-DNA in minutes through the expansion and convergence of distributively initiated bubbles; however, under physiological salt concentrations, unwinding activity is severely attenuated, while translocation is paradoxically stimulated. We, therefore, propose a model in which Sgs1—in complex with RPA and Top3-Rmi1—translocates on dsDNA via bubble migration during HJ dissolution in a mode phenomenologically distinct from its end-dependent, Dna2-associated helicase activity.

DNA Structure and Dynamics III

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Construction and Characterization of Cy3- or Cy5-Conjugated Hairpin Pyrrole/Imidazole Polyamides Binding to DNA in the Nucleosome

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N-Methylpyrrole (Py)-N-methylimidazole (Im) polyamides are small molecules that can recognize specific DNA sequences in the minor groove of B-form DNA with DNA recognition rules. Py favors the T, A, and C bases, excluding G and Im favors G. Lone pair of N3 in Im forms a hydrogen bond with 2 amino hydrogen of guanine. Anti-parallel pairings of Im/Py and Py/Im bind to the G•C and C•G sequence in DNA, respectively. Anti-parallel pairing of Py/Py binds to A•T and T•A degenerately. Aliphatic β -alanine (β) can be substituted for Py. Anti-parallel pairings of Py/ β and β /Py bind to A•T and T•A degenerately, and anti-parallel pairings of Im/ β and β /Im specify G•C and C•G, respectively. Recently, Py-Im polyamides have been conjugated with fluorophores and some of the fluorophores conjugate Py-Im polyamides could be used for specific DNA detection. In this study, we synthesized two Py-Im polyamide 1 and 2, which interact with the 145 bp DNA containing nucleosome positioning sequence 601. We conjugated cyanine dyes Cy3 or Cy5 with 1 or 2. In the absence of the target DNA, the fluorescent intensity from the fluorescence conjugate Py-Im polyamide diminished their fluorescence, compared with Cy3 or Cy5. In the presence of the target DNA or nucleosome, the fluorescent intensity from the fluorescence conjugate Py-Im polyamides increased. Furthermore, interestingly, FRET between Cy3-Py-Im polyamide and Cy5-Py-Im polyamide on nucleosome was observed. These results provide possibilities that the fluorescent conjugates of Py-Im polyamides can be used for characterization of the dynamic interactions within protein-DNA complexes.

3513-Pos Board B241

Extraction of Conventional Two-State Melting Temperature from DNA Oligomers with Significant Premelting Behavior

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The determination of some thermodynamic quantities of DNA, including melting temperature, may be confounded by premelt behavior. This is a significant problem for fluorescent observation of DNA melting and can be further exacerbated by the use of common dyes, which may actually increase deviation from the simple two-state melt model. Using contact-quenched fluorescently labeled DNA oligomers², we have developed a fitting function for fluorescent melting curves that may be used to extract the conventional 'two-state' melting temperature, even in the presence of significant premelt behavior of oligomers